Prestige Diagnostics
Clinical Diagnostics & Point of Care Products

ANTI HIV Ag/Ab 4th Gen Elisa

CAT NO	DESCRIPTION	PACK SIZE
EIAHV41	Anti HIV Ag/Ab 4th Gen Elisa	96 Tests
EIAHV42	Anti HIV Ag/Ab 4th Gen Elisa	480 Tests

Intended Use:

Anti HIV Ag/Ab Elisa is an Enzyme linked immunosorbent assay intended for the qualitative detection of antigens and/0r antibodies to HIV type (group M-O) and/0r type 2 in human serum or plasma samples. The method is also known as the 4th Generation Elisa assay for the detection of HIV. The kit can be used for the screening of blood donors and as an aid in the diagnosis of clinical conditions related to infection with HIV-1 and HIV-2 the etiological agents of AIDS. This reagent is for In vitro Diagnostic use only.

Summary and Principle:

The human immunodeficiency viruses type 1 and type 2 are the etiological agents of the acquired immunodeficiency syndrome (AIDS) and related conditions. HIV has been isolated from patients with AIDS, AIDS related complex (ARC) and from healthy individuals at high risk for AIDS. Infection with HIV is followed by an acute flu-like illness. This phase may remain unnoticed and the relationship to HIV infection may not be clear in many cases. The acute phase is typically followed by an asymptomatic carrier state, which progresses to clinical AIDS in about 50% of infected individuals within 10 years after seroconversion. Serological evidence of infection with HIV may be obtained by testing for presence of HIV antigens or antibodies in serum of individuals suspected for HIV infection. Antigens can generally be detected during both acute phase and the symptomatic phase of AIDS only. The Antibodies to HIV-1 and/or HIV-2 can be detected throughout virtually the whole infection period, starting at, or shortly after the acute phase and lasting till the end stage of AIDS. Apart from sexual transmission, the principal route of infection with HIV is blood transfusion. HIV can present both in cellular and cell-free fractions of human blood. Therefore, all donations of blood or plasma should be tested due to the risk of HIV transmission through contaminated blood.

The ELISA tests for detection of HIV infection are characterized with high sensitivity, specificity and simple operation procedure. These are most appropriate for testing of large numbers of specimens and currently, internationally available are hundreds of HIV tests used in routine blood screening or clinical diagnosis. Since the first HIV ELISA tests were commercially introduced in 1985, four more generations have been developed. The 1st generation tests were based on viral lysate antigens derived from viruses that are grown in human T-lymphocyte lines. The presence of traces of host cell components in which the virions have been propagated could lead to cross-contamination and thus to very high rates of false-positive results. With the cloning of the HIV genome, improved assays based on recombinant proteins and/or synthetic peptides (known as 2nd generation), became rapidly available. The utilization of biotechnology methods allow predominantly expression of the important immunoreactive regions of the proteins and also enabled the production of combined HIV-1/HIV-2 assays. The recombinant antigen could also be produced with considerably more purity and in large amounts, and they can be bond to solid-phase surface with much tighter control over protein ratios and concentrations. The first and second generations HIV kits were based on indirect ELISA method and could detect IgG antibodies only by enzyme-labeled anti-human IgG antibody. The third generation ELISA utilized double antigen "sandwich" method, again with antigens coated on solid phase polystyrene plates, but with antibodies detection achieved with the help of another enzyme-labeled antigen. The third generation assays could detect all antibodies in sample (IgG, IgM, etc.) which significantly increases the assay's sensitivity comparing to the previous generations. In addition, the detection of IgM antibodies that are present only during the early stages of infection, much shortens the antibody detection "window" period (the period of time in which there is no detectable antibody production), and compare to the second generation, "sandwich" tests could detect antibodies 11 days earlier. To reduce even further the antibody detection "window" period, 4th generation HIV ELISAs that could simultaneously detect HIV antigens (p24) and antibodies have been developed and are commercially available since 1998. With detection of p24, the 4th generation tests shorten the "window" period to 16 days, or compare to the 3rd generation, HIV infection could be detected 8 days earlier. HIV 1+2 Ag/Ab ELISA is a two-step incubation, "sandwich" enzyme immunoassay kit, which uses polystyrene microwell strips pre-coated with recombinant HIV antigens (recombinant HIV-1 gp41, gp120, and recombinant HIV-2 gp36) and anti-HIV (p24) antibodies. As a first step, biotinylated anti-HIV (p24) antibodies together with the patient's serum or plasma sample are added into the wells. During incubation, the specific HIV-1/2 antibodies if present in sample, will be captured inside the wells. Simultaneously, if HIV p24 antigen is present in sample, it will also be captured as a double antibody "sandwich" complex comprising of the coated antibodies-p24-biotinylated antibodies. The microwells are then washed to remove unbound serum proteins. The detection of the captured HIV p24 antigen-biotinylated antibody complex or HIV-1/2 antibodies is achieved during the second incubation step by adding of the enzyme Horseradish Peroxidase (HRP) which has been conjugated to second HIV 1+2 recombinant antigens and to avidin.

p24 detection: When p24 has been captured inside the wells, avidin will react with the biotin and attach HRP to the Ab-p24-Ab complex. HIV-1/2 antibody detection: When HIV-1/2 antibodies have been captured inside the wells, the HRP-conjugated antigens will bind to the captured antibodies forming Ag-Ab-Ag (HRP) "sandwich" immunocomplex.

The microwells are washed to remove unbound conjugate, and Chromogen solutions are added to the wells. In wells containing the Ag-Ab-Ag (HRP) and/or Ab-p24-Ab (HRP) "sandwich" immunocomplexes, the colourless chromogens are hydrolyzed by the bound HRP to a blue coloured product. The blue colour turns yellow after stopping the reaction with sulfuric acid. The degree of colour intensity can be measured and is proportional to the concentration of antibodies or p24 captured in the wells, and in the sample respectively. Wells containing samples negative for anti-HIV-1/2 or p24 remain colourless.

Reagent Composition: (for 96 and 480 test packs)

COMPONENT	SIZE	DESCRIPTION
Microwell Plate	1x96 wells (12x8 well plate) 5x96 wells	Each microwell is coated with recombinant HIV1/2 antigens and p24 antibodies. Place unused wells or strips in the provided plastic sealable bag together with the desiccant and store at 2-8°C. Once open the wells are stable for 1 month at 2-8°C.
Negative Control	1x1ml 3x1ml	Protein stabilized buffer tested non-reactive for HIV 1 / 2. Yellow in colour. Ready to use. Once open stable for 1 month at 2-8°C.
Positive Control 1	1x1ml 3x1ml	Protein stabilized buffer dilution of HIV 1 antibodies. Red coloured solution. Once open, stable for 1 month at 2-8°C.
Positive Control 2	1x1ml 3x1ml	Protein stabilized buffer dilution of HIV 2 antibodies. Red coloured solution. Once open, stable for 1 month at 2-8°C.
Positive Control 3	1x1ml 3x1ml	Red coloured liquid filled in a vial with blue cap. Protein stabilized buffer tested positive for p24 antigens.
HRP-Conjugate	1x12ml 5x`12ml	Red coloured liquid. HRP conjugated recombinant HIV 1+2 antigens. Once open, stable for one month at 2-8°C.
Biotin Conjugate	1x3.0ml 5x3.0ml	Blue coloured liquid in a white vial. Biotinylated anti HIV -24 antibodies diluted in a protein stabilized buffer. Ready to use as supplied. Once open stable for 4 weeks at 2-8°C.
Wash Buffer (20X)	1x50ml 2x100ml	PBS at pH 7.4. 20X concentrate. Once open, stable for one month at 2-8°C. The concentrate must be diluted 1 to 20 with distilled water before use. Once diluted it is stable at room temperature for a week or two weeks at 2-8°C.
Chromogen A	1x6ml 1x60ml	Urea peroxide solution. Ready to use. Once open, stable for one month at 2-8°C.
Chromogen B	1x6ml 1x60ml	TMB Solution. Ready to use. Once open, stable for one month at 2-8°C.
Stop Solution	1x6ml 1x60ml	Diluted Sulfuric acid solution (0.5M) Ready to use. Once open, stable for 1 month at 2-8°C.

Plastic Sealable bag, IFU and plate covers.

Materials required but not provided:

Distilled water or deionized water, disposable gloves and timer, appropriate waste containers for potentially contaminated materials, dispensing systems, disposable pipette tips, absorbent tissue or clean towel, dry bath incubator or water bath, plate reader, single wavelength 450nm or dual wavelength 450/630nm and microwell aspiration systems.

Specimen Collection:

1. No special patient preparation is required. Collect the specimen in accordance with normal laboratory practice. Either fresh serum or plasma specimens can be used with this assay. Blood collected by venepuncture should be allowed to clot naturally and completely – the serum/plasma must be separated from the clot as early as possible as to avoid haemolysis of the RBC. Care should be taken to ensure that the serum specimens are clear and not contaminated by microorganisms. Any visible particulate matters in the specimens should be removed by centrifugation at 3000 RPM for 20 minutes at room temperature or by filtration.
2. Plasma specimens collected into EDTA, sodium citrate or heparin can be tested, but highly lipaemic, icteric or haemolytic specimens should not be used as they give false results in the assay. Do not heat inactivate specimens. This can cause deterioration of the target analyte. Samples with visible microbial contamination should never be used.
3. The Prestige Anti HIV Ag/Ab Elisa assay is used only for testing individual serum or plasma samples. Do not use for testing cadaver samples, saliva, urine or other body fluids or pooled (mixed) blood.
4. Transportation and Storage: store specimens at 2-8°C. Specimens not required for assaying within 7 days should be stored at -20°C or lower. Multiple free thaw cycles should be avoided. For shipment, samples should be packaged and labelled in accordance with the existing local and international regulations for transportation of clinical samples and ethological agents.

Storage and Stability:

The contents of the kit will remain stable up to expiry date when stored at 2-8°C. Do not freeze. Keep all components tightly capped and without any contamination.

Precautions and Safety:

The Elisa assays are time and temperature sensitive. To avoid incorrect results, strictly follow the test procedure and do not modify them.

1. **Do not exchange reagents from different lots or use reagents from other commercially available kits. The components of the kit are precisely matched for optimal performance of the tests.**
2. **Make sure that all reagents are within the validity indicated on the kit box and of the same lot. Never use reagents beyond their expiry date stated on labels or boxes.**
3. **CAUTION - CRITICAL STEP: Allow the reagents and specimens to reach room temperature (18-30°C) before use. Shake reagent gently before use. Return at 2-8°C immediately after use.**
4. **Use only sufficient volume of sample as indicated in the procedure steps. Failure to do so, may cause in low sensitivity of the assay.**
5. **Do not touch the bottom exterior of the wells; fingerprints or scratches may interfere with the reading. When reading the results, ensure that the plate bottom is dry and there are no air bubbles inside the wells.**
6. **Never allow the microplate wells to dry after the washing step. Immediately proceed to the next step. Avoid the formation of air bubbles when adding the reagents.**
7. **Avoid assay steps long time interruptions. Assure same working conditions for all wells.**
8. **Calibrate the pipette frequently to assure the accuracy of samples/reagents dispensing. Use different disposal pipette tips for each specimen and reagents in order to avoid cross-contaminations.**
9. **Assure that the incubation temperature is 37°C inside the incubator.**
10. **When adding specimens, do not touch the well's bottom with the pipette tip.**
11. **When measuring with a plate reader, determine the absorbance at 450nm or at 450/630nm.**
12. **The enzymatic activity of the HRP-conjugate might be affected from dust and reactive chemical and substances like sodium hypochlorite, acids, alkalis etc. Do not perform the assay in the presence of these substances.**
13. **If using fully automated equipment, during incubation, do not cover the plates with the plate cover. The tapping out of the remainders inside the plate after washing, can also be omitted.**
14. **All specimens from human origin should be considered as potentially infectious. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety.**
15. **WARNING: Materials from human origin may have been used in the preparation of the Negative Control of the kit. These materials have been tested with tests kits with accepted performance and found negative for antibodies to HIV 1/2, HCV, TP and HBsAg. However, there is no analytical method that can assure that infectious agents in the specimens or reagents are completely absent. Therefore, handle reagents and specimens with extreme caution as if capable of transmitting infectious diseases. Bovine derived sera have been used for stabilizing of the positive and negative controls. Bovine serum albumin (BSA) and fetal calf sera (FCS) are derived from animals from BSE/TSE free-geographical areas.**
16. **Never eat, drink, smoke, or apply cosmetics in the assay laboratory. Never pipette solutions by mouth.**
17. **Chemical should be handled and disposed of only in accordance with the current GLP (Good Laboratory Practices) and the local or national regulations.**
18. **The pipette tips, vials, strips and specimen containers should be collected and autoclaved for not less than 2 hours at 121oC or treated with 10% sodium hypochlorite for 30 minutes to decontaminate before any further steps of disposal. Solutions containing sodium hypochlorite should never be autoclaved. MSDS available upon request.**
19. **Some reagents may cause toxicity, irritation, burns or have carcinogenic effects as raw materials. Contact with skin and the mucosa should be avoided but not limited to the following reagents: stop solution, chromogen reagents and the wash buffer.**
20. **The stop solution contains sulfuric acid. Use it with appropriate care. Wipe up spills, immediately and wash with water if comes into contact with the skin or the eyes.**
21. **Proclin 300 is used as preservative and can cause sensation of the skin. Wipe up spills immediately or wash with water if comes into contact with skin or eyes.**
22. **INDICATIONS OF INSTABILITY OR DETERIORATION OF THE REAGENTS: The values of positive and negative controls which are out of the indicated quality control range, are indicators of possible deterioration of the reagents and or operator or equipment errors. In such cases, the results should be considered as invalid and the samples must be retested. In case of consistently erroneous results and proven deterioration or**

instability of the reagents, immediately discard the reagents in use and use a new kit. Contact the local Prestige representative.

Procedure:

Reagent preparation:

Allow the reagents to reach room temperature (18-30ºC). Check the wash buffer concentration for the presence of salt crystals. If crystals have formed, re-solubilize by warming at 37ºC, until crystals dissolve. Dilute the wash buffer (20X) as indicated in the instructions for washing. Use distilled or deionized water and clean vessels to dilute the buffer. All other reagents are ready to use as supplied.

STEP 1

Preparation: Mark 3 wells as Negative controls (e.g. B1,C1,D1), 3 wells as Positive controls 1 and 2 (e.g. E1 for HIV 1, F1 for HIV 2 and G1 for HIV-Ag) and one Blank (e.g.A1 – taking care that neither the HRP conjugate nor any samples should be added to the blank well). If the results are read using a plate reader having dual wavelength (450 / 630nm) then the Blank well need not be used. Use the required number of strips for the test.

STEP 2

Addition of Biotin Conjugate: Add 20ul of Biotin Conjugate to all wells except the Blank well. Mix by tapping the plate gently.

STEP 3

Addition of the sample: Add 100ul of Positive control, Negative Control and Specimen into their respective wells except the Blank. (Note: Use a separate disposal pipette tip for each specimen, Negative control, Positive control to avoid cross contamination).

STEP 4

Incubation: Cover the plate with the plate cover and incubate for 60 minutes at 37ºC.

STEP 5

Washing: At the end of the incubation period, remove and discard the plate cover and contents of the microwells. Wash each well 5 times with diluted washing buffer. Each time allow the microwells to soak for 20 seconds. After the final washing cycle, turn down the plate onto a blotting paper or a clean towel and tap it to remove any residual buffer.

STEP 6

Addition of HRP Conjugate: Add 100ul of HRP Conjugate into each well except the Blank well.

STEP 7

Incubation: Cover the plate with the plate cover and incubate for 30 minutes at 37ºC.

STEP 8

Washing: At the end of the incubation period, remove and discard the plate cover. Wash each well 5 times with diluted washing buffer. Each time allow the microwells to soak for 20 seconds. After the final washing cycle, turn down the plate onto a blotting paper or a clean towel and tap it to remove any residual buffer.

STEP 9

Addition of the chromogen: Add 50ul of Chromogen A and 50ul of Chromogen B into each well including the blank. Incubate the plate at 37ºC for 30 minutes avoiding light. The enzymatic reaction between the chromogen solutions and the HRP conjugate produces blue colour in Positive controls and HIV 1 / 2 Positive samples.

STEP 10

Stopping the Reaction: Add 50ul of the Stop solution into each well and mix gently. Intensive yellow colour develops in the positive control and HIV 1 / 2 positive sample wells.

STEP 11

Measurement: Calibrate the plate reader with the Blank well and read the absorbance at 450nm. If a dual filter instrument is used, set the reference wavelength at 630nm. Calculate the cut off value and evaluate the results. (Note: Absorbances must be read within 10 minutes of adding the stop solution).

Instructions for Washing:

- To remove any effect washing on false positive reactions, a 5 automatic wash cycle is required with 350-400ul of diluted wash buffer used per well per wash. This helps in avoiding false positive reactions and a high background.
- To avoid cross-contamination of the plate with specimen or HRP conjugate, after incubation, do not discard the content of the wells but allow the plate washer to aspirate it automatically.
- Assure that the microplate washer liquid dispensing channels are not blocked or contaminated and sufficient volume of wash buffer is dispensed each time into the wells.
- In case of manual washing, we suggest to carry out 5 washing cycles, dispensing 350-400ul/well and aspirating the liquid 5 times. If poor results are observed with high background, increase washing cycles to soak time per well.
- Treat the liquid aspirated after the reaction from the wells with Sodium hypochlorite (at a concentration of 2.5%) for 24 hours before they are disposed off in the appropriate way.
- The concentrated wash buffer should be diluted 1:20 before use. If less than a whole plate is used, prepare the proportional volume of solution.

Calculation of results:

Each microplate should be considered separately when calculating and interpreting the results of the assay, regardless of the number of plates concurrently processed. The results are calculated by relating each specimen absorbance (A) with the cut off value (C.O) of the plate. If the cut off reading is based on single filter plate reader, the results should be calculated by subtracting the Blank well A from the absorbances of the specimens and the controls. In case the results are based on a dual filter plate reader, do not subtract the blank value A from the specimen and controls absorbances.

Calculation:

Cut off value (C.O) = Nc + 0.12

(Nc = the mean absorbance value for 3 negative controls)

Validation:

Blank well: the absorbance must be <0.080 at 450nm.

Positive Control: the absorbance must be >/= 0.800 at 450/630nm 0r at 450nm after blanking.

Negative control: the absorbance must be <0.100 at 450/630nm or at 450nm after blanking.

If one of the Negative control absorbances does not match the above criteria, this value should be discarded and a mean value should be calculated using the other two values. If more than one negative control absorbance does not meet the criteria, the test is invalid and must be re-tested.

Example:

Blank Value	A1: 0.025	450nm (blanking is required only when reading with a single filter)	
Negative control	B1	C1	D1
	0.020	0.012	0.016
Positive control	E1	F1	G1
	2.421	2.369	2.893

Calculation of Nc: (0.016+0.012+0.016)/3) = 0.016

Calculation of the cut off: 0.016 + 0.12 = 0.136

Interpretation of the results:

Negative Results: (A/CO <1) Specimens giving absorbance less than that of the Cut off value are negative for this assay. This indicates that the sample is non-reactive for HIV 1 / 2 antibodies and the patient is probably not infected with HIV 1 / 2 and the blood unit does not contain HIV 1 / 2 and could be transfused in case other infectious diseases markers are also absent.

Positive Results: (A/CO >/= 1) Specimens giving an absorbance equal to or greater than the cut off values are considered initially reactive, indicating that HIV 1 / 2 antibodies have been probably detected. All initially reactive samples should be re-tested with the same kit before final interpretation. Repeatedly reactive specimens can be considered positive for HIV ½ antibodies.

Borderline: (A/CO = 0.9 – 1.1) Specimens with absorbance to cut off ratio between 0.9 to 1.1 are considered borderline and retesting of these specimens in duplicates is required to confirm the initial results.

Follow up, confirmation and supplementary testing of any positive specimen with other analytical systems such as WB, PCR is required. Clinical diagnosis should not be established using a single result.

- After re-testing of the initially reactive samples, both wells show negative results (A/CO <0.9). These samples should be considered negative and the original result must be classified as false positive. As with many sensitive Elisa Assays, false positive results can occur due to several reasons, most of which are connected with, but not limited to inadequate washing step.
- After retesting in duplicates one or both wells show positive results. The final result of this specimen should be recorded as repeatedly positive. Repeatedly reactive specimens could be considered positive for antibodies to HIV 1 / 2 and the blood unit must be discarded.
- After re-testing in duplicates, samples with values close to the cut-off should be interpreted with caution and considered borderline samples, or uninterpretable for the time of testing.

Performance Characteristics:

Detailed Performance characteristics can be requested from the technical department of Prestige UK.

Specificity: Overall diagnostics Specificity is 99.90%.

Sensitivity: Overall sensitivity: 100%

Limitations:

- Positive results must be confirmed with another available method and interpreted in conjunction with the patient clinical information.
- Antigens may be undetectable during the early stages of the disease. Negative results are only an indication that the sample does not contain detectable levels of HIV 1 / 2 antibodies.
- If, after re-testing of the initially reactive specimens, the assay results are negative, these samples should be considered as non-repeatable and interpreted as negative. As with many sensitive Elisa assays, false positive reactions occur due to several reasons most of which are related to but not limited to inadequate washing step.
- The most common assay mistakes are: using kits beyond expiry dates, bad washing procedures, contaminated reagents, improper operation with equipment, sample collection issues.
- The prevalence of the marker will affect the assay's predictive values.
- This assay cannot be utilized to test pooled plasma. This kit can only be used with individual serum or plasma samples.
- This assay is a qualitative assay and the results cannot be used to measure antibody concentrations. This assay cannot be used to distinguish between infections with HIV 1 and HIV 2.

References:

1. Barre-Sinoussi, F et al., (1984) Isolation of a T-lymphotropic retrovirus from a patient at risk for acquiredimmunodeficiency syndrome (AIDS), Science, 220: 868-871.
2. Barbe, F.et al., (1994) Early detection of antibodies to HIV-1 by a third generation enzyme immunoassay. Ann. Biol. Clin. (Paris), 52: 341-345.
3. Constantine,. N., T. et al., (1993) Serologic test for the retroviruses: approaching a decade of evolution. AIDS, 7: 1-13Gnann JW et al. (1987) Science; 237: 1346-1349.

REF	Catalog number		Temperature limitation
	Consult instructions for use	LOT	Batch code
IVD	*In vitro* diagnostic medical device		Use by
	Manufacturer		

Prestige Diagnostics U.K. Ltd 40 Ballymena Business Centre, Galgorm, Co. Antrim, BT42 1FL, United Kingdom. Tel: +44 (0) 28 2564 2100

www.prestigediagnostics.co.uk info@prestigediagnostics.co.uk

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